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PATENT

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

MANCEBO et al.

Serial No. 10/043,649

Filed: January 10, 2002

For: *Cloning of a Novel Inhibitor of Antigen-receptor Signaling by a Retroviral-based Functional Screen*

Examiner: not yet assigned

Art Unit: 1645

CERTIFICATE OF MAILING

I hereby certify that this correspondence, including listed enclosures, is being deposited with the United States Postal Service as First Class Mail in an envelope addressed to: Commissioner for Patents, BOX SEQUENCE, Arlington , VA 22202 on:

Dated: 5 November 2002

Signed Mari Kleineidam

Mari Kleineidam

PRELIMINARY AMENDMENT AND STATEMENT RE SEQUENCE LISTING

Commissioner for Patents
U.S. Patent and Trademark Office
BOX SEQUENCE
Arlington, VA 22202

Sir:

This amendment is in response to the Notice to File Missing Parts of Nonprovisional Application mailed April 9, 2002. A petition for extension of time and the requisite fee, extending the period for reply by five months up to and including 9 November 2002, is being filed separately in this application under 37 CFR § 1.53(f) on 5 November 2002, making this a timely response. Copies of the Petition and the Transmittal of Missing Parts Under 37 CFR § 1.53(f) as filed 5 November 2002 are attached hereto.

Please amend the application as follows in adherence with rules 37 C.F.R. §§ 1.821-1.825:

IN THE SPECIFICATION

Please replace the paragraph beginning at page 3, line 36, with the following rewritten paragraph:

--In a preferred embodiment, the SLIM nucleic acid comprises a nucleic acid sequence having at least about 85%, more preferably at least about 90%, more preferably at least about 95%, more preferably at least about 98% identity to the nucleic acid sequence set forth in Figure 2A (SEQ ID NO:1). In a preferred embodiment, the SLIM nucleic acid comprises the nucleic acid sequence set forth in Figure 2A. In a preferred embodiment, the SLIM nucleic acid encodes a SLIM protein. --

Please replace the paragraph beginning at page 4, line 5, with the following rewritten paragraph:

--In a preferred embodiment, the SLIM protein comprises an amino acid sequence encoded by a nucleic acid sequence having at least about 85%, more preferably at least about 90%, more preferably at least about 95%, more preferably at least about 98% identity to the nucleic acid sequence set forth in Figure 2A (SEQ ID NO:1). In a preferred embodiment, the SLIM protein comprises an amino acid sequence encoded by the nucleic acid sequence set forth in Figure 2A. --

Please replace the paragraph beginning at page 4, line 11, with the following rewritten paragraph:

--Also provided herein are SLIM antisense nucleic acids which nucleic acids will hybridize under high stringency conditions to a SLIM nucleic acid comprising the nucleic acid sequence set forth in Figure 2A (SEQ ID NO:1). In a preferred embodiment, antisense nucleic acid comprises a nucleic acid sequence complementary to that set forth in Figure 2A or a fragment thereof. In a preferred embodiment, the SLIM antisense nucleic acid inhibits expression of SLIM protein encoded by SLIM nucleic acid. In a preferred embodiment, the SLIM antisense nucleic acid inhibits SLIM protein activity. In a preferred embodiment, the

SLIM antisense nucleic acid has at least one activity possessed by a SLIM protein variant described herein. --

Please replace the paragraph beginning at page 4, line 20, with the following rewritten paragraph:

--In a preferred embodiment, the SLIM protein comprises an amino acid sequence having at least about 85%, more preferably at least about 90%, more preferably at least about 95%, more preferably at least about 98% identity to the amino acid sequence set forth in Figure 2A (SEQ ID NO:1). In a preferred embodiment, the SLIM protein comprises the amino acid sequence set forth in Figure 2A. In a preferred embodiment, SLIM comprises an N-terminal SH2 domain, an N-terminal SH3 domain, an N-terminal myristylation sequence, and lacks a tyrosine kinase domain. Preferably, the SLIM protein also possesses one or more SLIM bioactivities described herein. --

Please replace the paragraph beginning at page 7, line 30, with the following rewritten paragraph:

-- Figure 2A Human SLIM cDNA and protein sequences (SEQ ID NOS:1-2). The putative myristylation site is shown in bold. Open and solid triangles indicate the boundaries of SH3 and SH2 domains, respectively. These sequence data are available at Genbank under accession no. AF326353. --

Please replace the paragraph beginning at page 7, line 34, with the following rewritten paragraph:

-- Figure 2B Alignment of human SLIM and SLAP

Identical amino acids are boxed and highlighted. Open and solid triangles indicate the boundaries of SH3 and SH2 domains, respectively. The overall amino acid similarity between SLIM (SEQ ID NO:2) and SLAP (SEQ ID NO:3) is 36%. In the SH2 and SH3 domains alone, the similarity between SLIM and SLAP is 48% and between SLIM and the Src family kinase Hck is 45%. --

Please replace the paragraph beginning at page 11, line 21, with the following rewritten paragraph:

--In one embodiment, the present invention provides SLIM nucleic acids comprising the nucleic acid sequence set forth in Figure 2A (SEQ ID NO:1). --

Please replace the paragraph beginning at page 11, line 24, with the following rewritten paragraph:

--In one embodiment, the present invention provides SLIM proteins comprising the amino acid sequence set forth in Figure 2A (SEQ ID NO:2). --

Please replace the paragraph beginning at page 11, line 34, with the following rewritten paragraph:

--A SLIM protein of the present invention may be identified in several ways. "Protein" in this sense includes proteins, polypeptides, and peptides. A SLIM nucleic acid or SLIM protein may be initially identified by substantial nucleic acid and/or amino acid sequence homology to the sequences shown in Figure 2A (SEQ ID NOS:1-2). Such homology can be based upon the overall nucleic acid or amino acid sequence. In addition, preferred SLIM proteins of the present invention comprise an SH2 domain, an SH3 domain, a myristylation sequence, and lack a tyrosine kinase domain. Preferred SLIM proteins of the present invention also possess SLIM protein activity as described below. SLIM proteins that bind to Cbl are especially preferred. In a preferred embodiment, SLIM proteins bind to Cbl in response to activation of antigen receptor. --

Please replace the paragraph beginning at page 12, line 12, with the following rewritten paragraph:

--As used herein, a protein is a "SLIM protein" if the overall homology of the protein sequence to the amino acid sequence shown in Figure 2A (SEQ ID NO:2) is preferably greater than about 75%, more preferably greater than about 80%, even more preferably greater than about 85% and most preferably greater than 90%. In some embodiments the homology will be as high as about 93 to 95 or 98%. Homology in this context means sequence similarity or

identity, with identity being preferred. This homology will be determined using standard techniques known in the art, including, but not limited to, the local homology algorithm of Smith & Waterman, Adv. Appl. Math. 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, J. Mol. Biol. 48:443 (1970), by the search for similarity method of Pearson & Lipman, PNAS USA 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Drive, Madison, WI), the Best Fit sequence program described by Devereux et al., Nucl. Acid Res. 12:387-395 (1984), preferably using the default settings, or by inspection. --

Please replace the paragraph beginning at page 13, line 16, with the following rewritten paragraph:

--The alignment may include the introduction of gaps in the sequences to be aligned. In addition, for sequences which contain either more or fewer amino acids than the protein shown in 2A (SEQ ID NO:2), it is understood that the percentage of homology will be determined based on the number of homologous amino acids in relation to the total number of amino acids. Thus, for example, homology of sequences shorter than that shown in the Figure, as discussed below, will be determined using the number of amino acids in the shorter sequence. --

Please replace the paragraph beginning at page 13, line 23, with the following rewritten paragraph:

--SLIM proteins of the present invention have structural homology to the known SLAP protein but are distinct therefrom. Comparison of the amino acid sequence of a preferred SLIM protein, which amino acid sequence is set forth in Figure 2A (SEQ ID NO:2), and the amino acid sequence of human SLAP, set forth at Genbank accession number NM_006748, reveals an overall sequence similarity of 36% and less than 50% similarity through the shared SH2 and SH3 domains (see Figure 2B (SEQ ID NOS:2-3) and Holland et. al., J. Exp. Med. 194:1263-1276, 2001). The C-terminal sequences of SLIM and SLAP proteins are divergent. Notably, it is the C-terminal domain of SLIM that mediates Cbl binding. --

Please replace the paragraph beginning at page 14, line 13, with the following rewritten paragraph:

--SLIM proteins may also be identified as being encoded by SLIM nucleic acids. Thus, SLIM proteins are encoded by nucleic acids that will hybridize to the sequence depicted in Figure 2A (SEQ ID NO:2), or its complement, as outlined herein. In a preferred embodiment, SLIM proteins provided herein are encoded by a SLIM nucleic acid comprising a nucleic acid sequence having at least about 75%, more preferably at least about 80%, more preferably at least about 85%, more preferably at least about 90%, more preferably at least about 95%, more preferably at least about 98% identity to the nucleic acid sequence set forth in Figure 2A (SEQ ID NO:1). --

Please replace the paragraph beginning at page 14, line 21, with the following rewritten paragraph:

--In a preferred embodiment, when the SLIM protein is to be used to generate antibodies, the SLIM protein must share at least one epitope or determinant with the full length protein shown in Figure 2A (SEQ ID NO:2). By "epitope" or "determinant" herein is meant a portion of a protein which will generate and/or bind an antibody. Thus, in most instances, antibodies made to a smaller SLIM protein will be able to bind to the full length protein. In a preferred embodiment, the epitope is unique; that is, antibodies generated to a unique epitope show little or no cross-reactivity. The term "antibody" includes antibody fragments, as are known in the art, including Fab Fab_2, single chain antibodies (Fv for example), chimeric antibodies, etc., either produced by the modification of whole antibodies or those synthesized de novo using recombinant DNA technologies. --

Please replace the paragraph beginning at page 15, line 4, with the following rewritten paragraph:

--In the case of the nucleic acid, the overall homology of the nucleic acid sequence is commensurate with amino acid homology but takes into account the degeneracy in the genetic

code and codon bias of different organisms. Accordingly, the nucleic acid sequence homology may be either lower or higher than that of the protein sequence. Thus the homology of the nucleic acid sequence as compared to the nucleic acid sequence of Figure 2A (SEQ ID NO:1) is preferably greater than 75%, more preferably greater than about 80%, particularly greater than about 85% and most preferably greater than 90%. In some embodiments the homology will be as high as about 93 to 95 or 98%.--

Please replace the paragraph beginning at page 15, line 19, with the following rewritten paragraph:

--In one embodiment, the nucleic acid homology is determined through hybridization studies. Thus, for example, nucleic acids which hybridize under high stringency to the nucleic acid sequences shown in Figure 2A (SEQ ID NO:1) or its complement is considered a SLIM gene. High stringency conditions are known in the art; see for example Maniatis et al., Molecular Cloning: A Laboratory Manual, 2d Edition, 1989, and Current Protocols in Molecular Biology, ed. Ausubel, et al., J. Wiley & Sons publ., New York, 1988, both of which are hereby incorporated by reference. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. An extensive guide to the hybridization of nucleic acids is found in Tijssen, Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Acid Probes, "Overview of principles of hybridization and the strategy of nucleic acid assays" (1993). Generally, stringent conditions are selected to be about 5-10°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength, pH and nucleic acid concentration) at which 50% of the probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at T_m, 50% of the probes are occupied at equilibrium). Stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (e.g. 10 to 50 nucleotides) and at least about 60°C for long probes (e.g.

greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. --

Please replace the paragraph beginning at page 16, line 13, with the following rewritten paragraph:

--The nucleic acid may be double stranded, single stranded, or contain portions of both double stranded or single stranded sequence. As will be appreciated by those in the art, the depiction of a single strand ("Watson") also defines the sequence of the other strand ("Crick"); thus the sequence depicted in Figure 2A (SEQ ID NO:1) also includes the complement of the sequence. By the term "recombinant nucleic acid" herein is meant nucleic acid, originally formed *in vitro*, in general, by the manipulation of nucleic acid by endonucleases, in a form not normally found in nature. Thus an isolated SLIM nucleic acid, in a linear form, or an expression vector formed *in vitro* by ligating DNA molecules that are not normally joined, are both considered recombinant for the purposes of this invention. It is understood that once a recombinant nucleic acid is made and reintroduced into a host cell or organism, it will replicate non-recombinantly, i.e. using the in vivo cellular machinery of the host cell rather than *in vitro* manipulations; however, such nucleic acids, once produced recombinantly, although subsequently replicated non-recombinantly, are still considered recombinant for the purposes of the invention. --

Please replace the paragraph beginning at page 18, line 18, with the following rewritten paragraph:

--Accordingly, in one aspect of the invention, SLIM variants that lack at least one SLIM bioactivity are provided. In a preferred embodiment, SLIM variants are unable to bind to Cbl. Such SLIM variants include SLIM proteins which lack the C-terminal domain required for binding to Cbl and for inhibition of antigen receptor-induced lymphocyte activation. In a preferred embodiment, such SLIM variants comprise amino acids 1-194 depicted Figure 2A (SEQ ID NO:2). In a preferred embodiment, such SLIM variants lack the amino acid sequence set forth by amino acids 195-261 in Figure 2A. In another preferred embodiment, SLIM variants that do not localize to the plasma membrane are provided. Such SLIM variants include SLIM

proteins that lack the N-terminal myristylation sequence that facilitates membrane localization and inhibition of antigen receptor-induced lymphocyte activation by SLIM protein. In a preferred embodiment, such a SLIM variant has a point mutation at the second amino acid residue (Gly) of the amino acid sequence set forth in Figure 2A. In another preferred embodiment, SLIM variants that are unable to bind to tyrosine phosphorylated SLIM binding partners, which include tyrosine kinases and phosphatases that are modulated by antigen receptor activation, are provided. Such SLIM variants include SLIM proteins that lack an N-terminal SH2 domain. In another preferred embodiment, SLIM variants that are unable to bind to SLIM binding partners comprising proline rich regions are provided. Such SLIM variants include SLIM proteins that lack an N-terminal SH3 domain. --

On page 64, immediately preceding the heading "CLAIMS," please insert the enclosed text entitled "SEQUENCE LISTING."

IN THE CLAIMS

Please replace the claims with the amended claim set that follows:

1. (Amended) A SLIM nucleic acid encoding a SLIM protein, comprising a nucleic acid sequence having at least about 90% identity to the nucleic acid sequence set forth in Figure 2A (SEQ ID NO:1), wherein said SLIM protein comprises an N-terminal myristylation sequence, an N-terminal SH2 domain, and an N-terminal SH3 domain and will bind to Cbl.

2. The SLIM nucleic acid according to Claim 1, wherein said SLIM protein lacks a tyrosine kinase domain.

3. (Amended) The SLIM nucleic acid according to Claim 2, further comprising the nucleic acid sequence set forth in Figure 2A (SEQ ID NO:1).

4. (Amended) A SLIM nucleic acid encoding a SLIM protein, comprising a nucleic acid sequence having at least about 90% identity to the nucleic acid sequence set forth in Figure 2A (SEQ ID NO:1), wherein said SLIM protein comprises an N-terminal myristylation sequence and an N-terminal SH2 domain and is unable to bind to Cbl.

5. (Amended) A SLIM nucleic acid encoding a SLIM protein, comprising a nucleic acid sequence encoding an amino acid sequence having at least about 90% identity to the amino acid sequence set forth in Figure 2A (SEQ ID NO:2).

6. (Amended) A SLIM protein, comprising an amino acid sequence having at least about 90% identity to the amino acid sequence set forth in Figure 2A (SEQ ID NO:2), wherein said SLIM protein comprises an N-terminal myristylation sequence, an N-terminal SH2 domain, and an N-terminal SH3 domain and will bind to Cbl.

7. (Amended) The SLIM protein according to Claim 6, further comprising the amino acid sequence set forth in Figure 2A (SEQ ID NO:2).

8. (Amended) A SLIM protein, comprising an amino acid sequence having at least about 90% identity to the amino acid sequence set forth in Figure 2A (SEQ ID NO:2), wherein said SLIM protein comprises an N-terminal myristylation sequence and an N-terminal SH2 domain and is unable to bind to Cbl.

9. (Amended) A method for screening for a bioactive agent capable of binding to SLIM, comprising:

a) contacting a SLIM protein and a candidate agent; and

b) determining the binding of candidate bioactive agent to SLIM protein;

wherein said SLIM protein comprises an amino acid sequence having at least about 95% identity to the amino acid sequence set forth in Figure 2A (SEQ ID NO:2).

10. (Amended) A method for screening for a bioactive agent capable of modulating SLIM binding, comprising:

a) combining a SLIM protein, a candidate bioactive agent and Cbl; and

b) determining the binding of Cbl to SLIM in the presence of candidate bioactive agent;

wherein said SLIM protein comprises an amino acid sequence having at least about 95% identity to the amino acid sequence set forth in Figure 2A (SEQ ID NO:2) and wherein said SLIM protein will bid to Cbl in the absence of candidate bioactive agent.

11. (Amended) A method for screening for a bioactive agent capable of modulating lymphocyte activation, comprising:

a) contacting a candidate bioactive agent to a lymphocyte comprising a recombinant nucleic acid encoding a SLIM protein;

b) inducing activation of said lymphocyte; and

c) determining the activation of said lymphocyte in the presence and absence of said candidate bioactive agent;

wherein said SLIM protein comprises an amino acid sequence having at least about 95% identity to the amino acid sequence set forth in Figure 2A (SEQ ID NO:2), and wherein a difference in the activation of said lymphocyte in the presence and absence of said candidate bioactive agent indicates that said candidate bioactive agent is capable of modulating lymphocyte activation.

12. (Amended) The method according to Claim 11, wherein said SLIM protein comprises the amino acid sequence set forth in Figure 2A (SEQ ID NO:2).

13. The method according to Claim 11, wherein lymphocyte activation is done by activating antigen receptor in said lymphocyte.

14. The method according to Claim 11, wherein determining the activation of said lymphocyte comprises determining the activity of a nuclear factor in activated T cells (NFAT) responsive promoter.

15. The method according to Claim 11, wherein determining the activation of said lymphocyte comprises determining the expression of CD69.

16. (Amended) A method for screening for a bioactive agent capable of modulating the ubiquitination of a Cbl target protein, comprising:

a) combining SLIM, Cbl, ubiquitin or polyubiquitin, and a Cbl target protein; and

b) determining the level of ubiqitination of Cbl target protein in the presence and absence of candidate bioactive agent;

wherein said SLIM protein comprises an amino acid sequence having at least about 95% identity to the amino acid sequence set forth in Figure 2A (SEQ ID NO:2) and will bind to Cbl and Cbl target protein in the absence of candidate agent, wherein a change in the level of ubiquitination of Cbl target protein in the presence of candidate agent indicates that said candidate bioactive agent is capable of modulating the ubiquitination of a Cbl target protein.

REMARKS

The specification and claims have been amended to include a Sequence Listing and proper reference to the sequences therein and to correct minor typographical errors. A "clean" version of the now-pending claim set is provided above. Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "Version with markings to show changes made."

Entry of this amendment is respectfully requested. The amendments are made in adherence with 37 C.F.R. § 1.821-1.825. This amendment is accompanied by a floppy disk

containing the above named substitute sequence listing, SEQUENCE ID NUMBERS 1-3 in computer readable form, and a paper copy of the sequence information. The information contained in the computer readable disk is identical to that of the paper copy. This amendment contains no new matter. Applicant submits that this amendment, the accompanying computer readable sequence listing, and the paper copy thereof serve to place this application in a condition of adherence to the rules 37 C.F.R. §§ 1.821-1.825.

Please direct any calls in connection with this application to the undersigned at (415) 781-1989.

Respectfully submitted,

DORSEY & WHITNEY LLP

Dated: 4/5/02

Four Embarcadero Center
Suite 3400
San Francisco, CA 94111
(415) 781-1989

Robin M. Silva, Reg. No. 38,304
Filed under 37 C.F.R. Section 1.34(a)

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION

Paragraph beginning at page 3, line 36, has been amended as follows:

--In a preferred embodiment, the SLIM nucleic acid comprises a nucleic acid sequence having at least about 85%, more preferably at least about 90%, more preferably at least about 95%, more preferably at least about 98% identity to the nucleic acid sequence set forth in Figure ~~2~~ 2A (SEQ ID NO:1). In a preferred embodiment, the SLIM nucleic acid comprises the nucleic acid sequence set forth in Figure ~~2~~ 2A. In a preferred embodiment, the SLIM nucleic acid encodes a SLIM protein. --

Paragraph beginning at page 4 line 5 has been amended as follows:

--In a preferred embodiment, the SLIM protein comprises an amino acid sequence encoded by a nucleic acid sequence having at least about 85%, more preferably at least about 90%, more preferably at least about 95%, more preferably at least about 98% identity to the nucleic acid sequence set forth in Figure ~~2~~ 2A (SEQ ID NO:1). In a preferred embodiment, the SLIM protein comprises an amino acid sequence encoded by the nucleic acid sequence set forth in Figure ~~2~~ 2A. --

Paragraph beginning at page 4, line 11, has been amended as follows:

--Also provided herein are SLIM antisense nucleic acids which nucleic acids will hybridize under high stringency conditions to a SLIM nucleic acid comprising the nucleic acid sequence set forth in Figure ~~2~~ 2A (SEQ ID NO:1). In a preferred embodiment, antisense nucleic acid comprises a nucleic acid sequence complementary to that set forth in Figure ~~2~~ 2A or a fragment thereof. In a preferred embodiment, the SLIM antisense nucleic acid inhibits expression of SLIM protein encoded by SLIM nucleic acid. In a preferred embodiment, the SLIM antisense nucleic acid inhibits SLIM protein activity. In a preferred embodiment, the SLIM antisense nucleic acid has at least one activity possessed by a SLIM protein variant described herein. --

Paragraph beginning at page 4, line 20, has been amended as follows:

--In a preferred embodiment, the SLIM protein comprises an amino acid sequence having at least about 85%, more preferably at least about 90%, more preferably at least about 95%, more preferably at least about 98% identity to the amino acid sequence set forth in Figure ~~2~~ 2A (SEQ ID NO:1). In a preferred embodiment, the SLIM protein comprises the amino acid sequence set forth in Figure ~~2~~ 2A. In a preferred embodiment, SLIM comprises an N-terminal SH2 domain, an N-terminal SH3 domain, an N-terminal myristylation sequence, and lacks a tyrosine kinase domain. Preferably, the SLIM protein also possesses one or more SLIM bioactivities described herein. --

Paragraph beginning at page 7, line 30, has been amended as follows:

-- Figure 2A Human SLIM cDNA and protein sequences (SEQ ID NOS:1-2). The putative myristylation site is shown in bold. Open and solid triangles indicate the boundaries of SH3 and SH2 domains, respectively. These sequence data are available at Genbank under accession no. AF326353. --

Paragraph beginning at page 7, line 34, has been amended as follows:

-- Figure 2B Alignment of human SLIM and SLAP

Identical amino acids are boxed and highlighted. Open and solid triangles indicate the boundaries of SH3 and SH2 domains, respectively. The overall amino acid similarity between SLIM (SEQ ID NO:2) and SLAP (SEQ ID NO:3) is 36%. In the SH2 and SH3 domains alone, the similarity between SLIM and SLAP is 48% and between SLIM and the Src family kinase Hck is 45%. --

Paragraph beginning at page 11, line 21, has been amended as follows:

--In one embodiment, the present invention provides SLIM nucleic acids comprising the nucleic acid sequence set forth in Figure ~~2~~ 2A (SEQ ID NO:1). --

Paragraph beginning at page 11, line 24, has been amended as follows:

--In one embodiment, the present invention provides SLIM proteins comprising the amino acid sequence set forth in Figure ~~2~~ 2A (SEQ ID NO:2). --

Paragraph beginning at page 11, line 34, has been amended as follows:

--A SLIM protein of the present invention may be identified in several ways. "Protein" in this sense includes proteins, polypeptides, and peptides. A SLIM nucleic acid or SLIM protein

may be initially identified by substantial nucleic acid and/or amino acid sequence homology to the sequences shown in Figure ~~2~~ 2A (SEQ ID NOS:1-2). Such homology can be based upon the overall nucleic acid or amino acid sequence. In addition, preferred SLIM proteins of the present invention comprise an SH2 domain, an SH3 domain, a myristylation sequence, and lack a tyrosine kinase domain. Preferred SLIM proteins of the present invention also possess SLIM protein activity as described below. SLIM proteins that bind to Cbl are especially preferred. In a preferred embodiment, SLIM proteins bind to Cbl in response to activation of antigen receptor. --

Paragraph beginning at page 12, line 12, has been amended as follows:

--As used herein, a protein is a "SLIM protein" if the overall homology of the protein sequence to the amino acid sequence shown in Figure ~~2~~ 2A (SEQ ID NO:2) is preferably greater than about 75%, more preferably greater than about 80%, even more preferably greater than about 85% and most preferably greater than 90%. In some embodiments the homology will be as high as about 93 to 95 or 98%. Homology in this context means sequence similarity or identity, with identity being preferred. This homology will be determined using standard techniques known in the art, including, but not limited to, the local homology algorithm of Smith & Waterman, Adv. Appl. Math. 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, J. Mol. Biol. 48:443 (1970), by the search for similarity method of Pearson & Lipman, PNAS USA 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Drive, Madison, WI), the Best Fit sequence program described by Devereux et al., Nucl. Acid Res. 12:387-395 (1984), preferably using the default settings, or by inspection. --

Paragraph beginning at page 13, line 16, has been amended as follows:

--The alignment may include the introduction of gaps in the sequences to be aligned. In addition, for sequences which contain either more or fewer amino acids than the protein shown in ~~2~~ 2A (SEQ ID NO:2), it is understood that the percentage of homology will be determined based on the number of homologous amino acids in relation to the total number of amino acids. Thus, for example, homology of sequences shorter than that shown in the Figure, as discussed below, will be determined using the number of amino acids in the shorter sequence. --

Paragraph beginning at page 13, line 23, has been amended as follows:

--SLIM proteins of the present invention have structural homology to the known SLAP protein but are distinct therefrom. Comparison of the amino acid sequence of a preferred SLIM protein, which amino acid sequence is set forth in Figure ~~2~~ 2A (SEQ ID NO:2), and the amino acid sequence of human SLAP, set forth at Genbank accession number NM_006748, reveals an overall sequence similarity of 36% and less than 50% similarity through the shared SH2 and SH3 domains (see Figure ~~2~~ 2B (SEQ ID NOS:2-3) and Holland et. al., J. Exp. Med. 194:1263-1276, 2001). The C-terminal sequences of SLIM and SLAP proteins are divergent. Notably, it is the C-terminal domain of SLIM that mediates Cbl binding. --

Paragraph beginning at page 14, line 13, has been amended as follows:

--SLIM proteins may also be identified as being encoded by SLIM nucleic acids. Thus, SLIM proteins are encoded by nucleic acids that will hybridize to the sequence depicted in Figure ~~2~~ 2A (SEQ ID NO:2), or its complement, as outlined herein. In a preferred embodiment, SLIM proteins provided herein are encoded by a SLIM nucleic acid comprising a nucleic acid sequence having at least about 75%, more preferably at least about 80%, more preferably at least about 85%, more preferably at least about 90%, more preferably at least about 95%, more preferably at least about 98% identity to the nucleic acid sequence set forth in ~~FIGURE 2~~ Figure 2A (SEQ ID NO:1). --

Paragraph beginning at page 14, line 21, has been amended as follows:

--In a preferred embodiment, when the SLIM protein is to be used to generate antibodies, the SLIM protein must share at least one epitope or determinant with the full length protein shown in Figure ~~2~~ 2A (SEQ ID NO:2). By "epitope" or "determinant" herein is meant a portion of a protein which will generate and/or bind an antibody. Thus, in most instances, antibodies made to a smaller SLIM protein will be able to bind to the full length protein. In a preferred embodiment, the epitope is unique; that is, antibodies generated to a unique epitope show little or no cross-reactivity. The term "antibody" includes antibody fragments, as are known in the art, including Fab Fab_2, single chain antibodies (Fv for example), chimeric antibodies, etc., either produced by the modification of whole antibodies or those synthesized de novo using recombinant DNA technologies. --

Paragraph beginning at page 15, line 4, has been amended as follows:

--In the case of the nucleic acid, the overall homology of the nucleic acid sequence is commensurate with amino acid homology but takes into account the degeneracy in the genetic code and codon bias of different organisms. Accordingly, the nucleic acid sequence homology may be either lower or higher than that of the protein sequence. Thus the homology of the nucleic acid sequence as compared to the nucleic acid sequence of Figure ~~2~~ 2A (SEQ ID NO:1) is preferably greater than 75%, more preferably greater than about 80%, particularly greater than about 85% and most preferably greater than 90%. In some embodiments the homology will be as high as about 93 to 95 or 98%.--

Paragraph beginning at page 15, line 19, has been amended as follows:

--In one embodiment, the nucleic acid homology is determined through hybridization studies. Thus, for example, nucleic acids which hybridize under high stringency to the nucleic acid sequences shown in Figure ~~2~~ 2A (SEQ ID NO:1) or its complement is considered a SLIM gene. High stringency conditions are known in the art; see for example Maniatis et al., Molecular Cloning: A Laboratory Manual, 2d Edition, 1989, and Current Protocols in Molecular Biology, ed. Ausubel, et al., J. Wiley & Sons publ., New York, 1988, both of which are hereby incorporated by reference. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. An extensive guide to the hybridization of nucleic acids is found in Tijssen, Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Acid Probes, "Overview of principles of hybridization and the strategy of nucleic acid assays" (1993). Generally, stringent conditions are selected to be about 5-10°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength, pH and nucleic acid concentration) at which 50% of the probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at T_m, 50% of the probes are occupied at equilibrium). Stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (e.g. 10 to 50 nucleotides) and at least about 60°C for long probes (e.g.

greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. --

Paragraph beginning at page 16, line 13, has been amended as follows:

--The nucleic acid may be double stranded, single stranded, or contain portions of both double stranded or single stranded sequence. As will be appreciated by those in the art, the depiction of a single strand ("Watson") also defines the sequence of the other strand ("Crick"); thus the sequence depicted in Figure ~~2~~ 2A (SEQ ID NO:1) also includes the complement of the sequence. By the term "recombinant nucleic acid" herein is meant nucleic acid, originally formed *in vitro*, in general, by the manipulation of nucleic acid by endonucleases, in a form not normally found in nature. Thus an isolated SLIM nucleic acid, in a linear form, or an expression vector formed *in vitro* by ligating DNA molecules that are not normally joined, are both considered recombinant for the purposes of this invention. It is understood that once a recombinant nucleic acid is made and reintroduced into a host cell or organism, it will replicate non-recombinantly, i.e. using the in vivo cellular machinery of the host cell rather than *in vitro* manipulations; however, such nucleic acids, once produced recombinantly, although subsequently replicated non-recombinantly, are still considered recombinant for the purposes of the invention. --

Paragraph beginning at page 18, line 18, has been amended as follows:

--Accordingly, in one aspect of the invention, SLIM variants that lack at least one SLIM bioactivity are provided. In a preferred embodiment, SLIM variants are unable to bind to Cbl. Such SLIM variants include SLIM proteins which lack the C-terminal domain required for binding to Cbl and for inhibition of antigen receptor-induced lymphocyte activation. In a preferred embodiment, such SLIM variants comprise amino acids 1-194 depicted Figure ~~2~~ 2A (SEQ ID NO:2). In a preferred embodiment, such SLIM variants lack the amino acid sequence set forth by amino acids 195-261 in Figure ~~2~~ 2A. In another preferred embodiment, SLIM variants that do not localize to the plasma membrane are provided. Such SLIM variants include SLIM proteins that lack the N-terminal myristylation sequence that facilitates membrane localization and inhibition of antigen receptor-induced lymphocyte activation by SLIM protein. In a preferred embodiment, such a SLIM variant has a point mutation at the second amino acid

residue (Gly) of the amino acid sequence set forth in Figure ~~2~~ 2A. In another preferred embodiment, SLIM variants that are unable to bind to tyrosine phosphorylated SLIM binding partners, which include tyrosine kinases and phosphatases that are modulated by antigen receptor activation, are provided. Such SLIM variants include SLIM proteins that lack an N-terminal SH2 domain. In another preferred embodiment, SLIM variants that are unable to bind to SLIM binding partners comprising proline rich regions are provided. Such SLIM variants include SLIM proteins that lack an N-terminal SH3 domain. --

On page 64, immediately preceding the heading "CLAIMS," the enclosed text entitled "SEQUENCE LISTING" was inserted into the specification.

IN THE CLAIMS

The Claims have been amended as follows:

1. (Amended) A SLIM nucleic acid encoding a SLIM protein, comprising a nucleic acid sequence having at least about 90% identity to the nucleic acid sequence set forth in Figure ~~2~~ 2A (SEQ ID NO:1), wherein said SLIM protein comprises an N-terminal myristylation sequence, an N-terminal SH2 domain, and an N-terminal SH3 domain and will bind to Cbl.—

3. (Amended) The SLIM nucleic acid according to Claim 2, further comprising the nucleic acid sequence set forth in Figure ~~2~~ 2A (SEQ ID NO:1).

4. (Amended) A SLIM nucleic acid encoding a SLIM protein, comprising a nucleic acid sequence having at least about 90% identity to the nucleic acid sequence set forth in Figure ~~2~~ 2A (SEQ ID NO:1), wherein said SLIM protein comprises an N-terminal myristylation sequence and an N-terminal SH2 domain and is unable to bind to Cbl.

5. (Amended) A SLIM nucleic acid encoding a SLIM protein, comprising a nucleic acid sequence encoding an amino acid sequence having at least about 90% identity to the amino acid sequence set forth in Figure ~~2~~ 2A (SEQ ID NO:2).

6. (Amended) A SLIM protein, comprising an amino acid sequence having at least about 90% identity to the amino acid sequence set forth in Figure ~~2~~ 2A (SEQ ID NO:2), ~~wherien~~ wherein said SLIM protein comprises an N-terminal myristylation sequence, an N-terminal SH2 domain, and an N-terminal SH3 domain and will bind to Cbl.

7. (Amended) The SLIM protein according to Claim 6, further comprising the amino acid sequence set forth in Figure ~~2~~ 2A (SEQ ID NO:2).

8. (Amended) A SLIM protein, comprising an amino acid sequence having at least about 90% identity to the amino acid sequence set forth in Figure ~~2~~ 2A (SEQ ID NO:2), wherein said SLIM protein comprises an N-terminal myristylation sequence and an N-terminal SH2 domain and is unable to bind to Cbl.

9. (Amended) A method for screening for a bioactive agent capable of binding to SLIM, comprising:

a) contacting a SLIM protein and a candidate agent; and

b) determining the binding of candidate bioactive agent to SLIM protein;

wherein said SLIM protein comprises an amino acid sequence having at least about 95% identity to the amino acid sequence set forth in Figure ~~2~~ 2A (SEQ ID NO:2).

10. (Amended) A method for screening for a bioactive agent capable of modulating SLIM binding, comprising:

a) combining a SLIM protein, a candidate bioactive agent and Cbl; and

b) determining the binding of Cbl to SLIM in the presence of candidate bioactive agent;

wherein said SLIM protein comprises an amino acid sequence having at least about 95% identity to the amino acid sequence set forth in Figure ~~2~~ 2A (SEQ ID NO:2) and wherein said SLIM protein will bid to Cbl in the absence of candidate bioactive agent.

11. (Amended) A method for screening for a bioactive agent capable of modulating lymphocyte activation, comprising:

a) contacting a candidate bioactive agent to a lymphocyte comprising a recombinant nucleic acid encoding a SLIM protein;

b) inducing activation of said lymphocyte; and

c) determining the activation of said lymphocyte in the presence and absence of said candidate bioactive agent;

wherein said SLIM protein comprises an amino acid sequence having at least about 95% identity to the amino acid sequence set forth in Figure ~~2~~ 2A (SEQ ID NO:2), and wherein a difference in the activation of said lymphocyte in the presence and absence of said candidate bioactive agent indicates that said candidate bioactive agent is capable of modulating lymphocyte activation.

12. (Amended) The method according to Claim 11, wherein said SLIM protein comprises the amino acid sequence set forth in Figure ~~2~~ 2A (SEQ ID NO:2).—

16. (Amended) A method for screening for a bioactive agent capable of modulating the ubiquitination of a Cbl target protein, comprising:

a) combining SLIM, Cbl, ubiquitin or polyubiquitin, and a Cbl target protein; and

b) determining the level of ubiqitination of Cbl target protein in the presence and absence of candidate bioactive agent;

wherein said SLIM protein comprises an amino acid sequence having at least about 95% identity to the amino acid sequence set forth in Figure ~~2~~ 2A (SEQ ID NO:2) and will bind to Cbl and Cbl target protein in the absence of candidate agent, wherein a change in the level of

ubiquitination of Cbl target protein in the presence of candidate agent indicates that said candidate bioactive agent is capable of modulating the ubiquitination of a Cbl target protein.